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# Effect of nutrient loading on biogeochemical and microbial processes in a New England salt marsh

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**Abstract** Coastal marshes represent an important transitional zone between uplands and estuaries. One important function of marshes is to assimilate nutrient inputs from uplands, thus providing a buffer for anthropogenic nutrient loads. We examined the effects of nitrogen (N) and phosphorus (P) fertilization on biogeochemical and microbial processes during the summer growing season in a *Spartina patens* (Aiton (Muhl.)) marsh in the Narragansett Bay National Estuarine Research Reserve on Prudence Island (RI). Quadruplicate 1 m<sup>2</sup> plots were fertilized with N and P additions, N-only, P-only, or no additions. N-only addition significantly stimulated bacterial production and increased pore water NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations. Denitrification rates ranged

from 0 to 8 mmol m<sup>-2</sup> day<sup>-1</sup>. Fertilization had no apparent effect on soil oxygen consumption or denitrification measured in the summer in intact cores due to high core-to-core variation. P fertilization led to increased pore water dissolved inorganic phosphorus (DIP) concentrations and increased DIP release from soils. In contrast the control and N-only treatments had significant DIP uptake across the soil-water interface. The results suggest that in the summer fertilization has no apparent effect on denitrification rates, stimulates bacterial productivity, enhances pore water nutrient concentrations and alters some nutrient fluxes across the marsh surface.

**Keywords** Bacterial production · Fertilization · Denitrification · Nitrogen · Phosphorus · Salt marsh

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## Introduction

Eutrophication resulting from increased anthropogenic nutrient loading is a serious concern nationally and internationally (NRC 2000). Salt marshes are at the interface between the uplands and the estuary and are frequently the first recipients of high nutrient runoff via overland or groundwater flow (Page 1995; Tobias et al. 2001). In estuaries with high nutrient concentrations, tidal exchange between the estuary and marsh is

another source of nutrient enrichment. Studies examining the response of salt marsh vegetation to fertilization have had consistent results, from the first studies in Great Sippewisset marsh (Valiela et al. 1975) and Delaware marshes (Sullivan and Daiber 1974) through the present day (Boyer et al. 2001; Sundareshwar et al. 2003; Wigand et al. 2004a). Most studies have shown nitrogen limitation of aboveground marsh production (Sullivan and Daiber 1974; Valiela et al. 1975; Buresh et al. 1980; DeLaune and Patrick Jr 1980; Boyer et al. 2001; Wigand et al. 2004a), although phosphorus limitation can occur secondarily with greater marsh biomass in N + P treatments compared to N-only (van Wijnen and Bakker 1999; Sundareshwar et al. 2003).

The effects of fertilization on bacterial production and nutrient cycling in marsh soils are not well studied and the results of the studies to date are not as consistent as the plant response. Fertilization enhanced soil respiration in a South Carolina *Spartina alterniflora* (Lois.) marsh because microbial decomposers appeared to be nutrient limited (Morris and Bradley 1999). Phosphorus additions stimulated bacterial biomass, bacterial production, and pore water alkaline phosphatase activity suggesting phosphorus limitation of bacterial growth (Sundareshwar et al. 2003). Denitrification was enhanced in plots fertilized with inorganic nutrients (Teal and Howes 2000). Fertilization with organic nitrogen or sewage sludge enhanced denitrification in New England salt marshes (Kaplan 1977; Hamersley and Howes 2005) and inhibited it in Georgia salt marshes (Sherr and Payne 1981). Denitrification potential was inhibited by phosphorus additions to soils from control plots (Sundareshwar et al. 2003). Nitrogen fixation was reduced in fertilized marshes compared to controls (Van Raalte et al. 1974; Sundareshwar et al. 2003).

Most fertilization studies to date have focused on *S. alterniflora* with little work in *S. patens* marshes. *S. patens* is often dominant in the upper salt marsh, although it also occurs in saline flats, low dunes, and tidal shores from coastal, south-west Newfoundland and eastern Quebec, south to Virginia and Florida, and west to eastern Texas. In New England estuaries, *S. patens* is the dominant plant in the high marsh zone, where tidal

inundation usually occurs during spring tides. The relatively infrequent flooding leads to reduced removal of wrack and greater incorporation of organic matter into soils compared to the low marsh zone where *S. alterniflora* is dominant. *S. alterniflora* decays about twice as fast as *S. patens* (Buschbaum et al. 1991). This critical difference in organic matter cycling between *S. alterniflora* and *S. patens* marshes may also manifest as a differential response to fertilization between these marshes. We anticipate that *S. patens* marshes should be particularly sensitive to fertilization by enhancing the accumulation of organic material and the microbially mediated processing of this material. *S. patens* is endomycorrhizal, that is it harbors fungal symbionts in the roots (Cooke et al. 1993), and these root-fungi associations have been demonstrated to facilitate primarily phosphorus uptake in many land plants (Allen 1991; Smith and Read 1997). In addition, researchers have shown that endomycorrhizal *S. patens* also indirectly facilitate nitrogen fixation in the marsh soil possibly due to an increased release of organic exudates in the rhizosphere (Burke 2001; Burke et al. 2002a). When phosphorus is enriched in salt marsh plots, it may disrupt the endomycorrhizal symbiosis of *S. patens*, and cause a subsequent inhibition of the root fungal symbionts and diminish the release of organic exudates and nitrogen fixation (Wigand et al. 2004a).

This study examined how biogeochemical and microbial processes in a *S. patens* marsh were affected by fertilization with nitrogen and phosphorus. Measurements were made of bacterial abundance and production (leucine incorporation),  $N_2$ ,  $O_2$ , and inorganic nutrient ( $NH_4^+$ , DIP,  $NO_3^-$ ,  $NO_2^-$ ) fluxes across the marsh surface and pore water nutrients. An earlier study at this same site reported that fertilization with nitrogen, but not phosphorus led to an increase in above-ground plant biomass and higher concentrations of N, P, and C in the above-ground tissues (Wigand et al. 2004a). In the present study, we hypothesized that the increased plant production in the nitrogen-fertilized plots would result in enhanced mineralization (the breakdown of organic nitrogen to  $NH_4^+$ ) and other nitrogen transformations, such as nitrification (the oxidation

of  $\text{NH}_4^+$  to  $\text{NO}_3^-$ ) and denitrification (the reduction of  $\text{NO}_3^-$  to dinitrogen gas). Further, we hypothesized that P fertilization would result in an alteration of nutrient exchanges in the *S. patens* rhizosphere, in part, because of inhibition of root fungal symbionts and disruption of mycorrhizal-mediated P uptake and N-fixation leading to increased competition between plants and bacteria for nitrogen.

## Methods

### Study site

This study was conducted in a *S. patens* marsh near the Nags Creek ( $41^\circ 37.546' \text{N}$  and  $71^\circ 19.223' \text{W}$ ) in the Narragansett Bay National Estuarine Research Reserve on Prudence Island, RI. Sixteen  $1 \text{ m}^2$  plots were established with a  $2 \times 2$  factorial design with nitrogen and phosphorus as the treatments. Plots were randomly located in vegetated patches at least 3 m apart. When the randomization process placed a plot in a bare patch, the plot was moved to the nearest vegetated patch. At low tide, dissolved  $\text{Ca}(\text{NO}_3)_2$  and  $\text{P}_2\text{O}_5$  were sprinkled on the soil surface twice per month for the growing season, May–August, but monthly thereafter. Fertilizer application rates were  $2 \text{ g N m}^{-2}$  and  $0.2 \text{ g P m}^{-2}$  with a total addition of  $80 \text{ g N m}^{-2}$  and  $8 \text{ g P m}^{-2}$  for the entire 28 months of the experiment (from May 2000 to September 2002).

### Bacterial abundance and production

During July 2002, three 2.5 cm core samples of soil were taken from each plot and the top 1 cm was pooled and homogenized. Soil bacterial abundance was determined by collecting duplicate samples ( $0.1 \text{ cm}^3$ ) from the homogenized soil, which were placed in pre-weighed micro-centrifuge tubes and re-weighed. The samples were fixed with 1 ml of 2% formaldehyde in seawater (pre-filtered through a  $0.2 \mu\text{m}$  syringe filter) and stored at  $4^\circ\text{C}$  until analysis within 3 weeks. Samples were sonicated with a Branson® micro-tip sonifier (Model 250, setting #2) with 10–15 1-s bursts. Aliquots ( $50\text{--}100 \mu\text{l}$ ) of the slurry were

removed with a wide bore pipet, further diluted into 1 ml of phosphate buffered saline. A  $100 \mu\text{l}$  aliquot of this diluted sample was stained with DAPI ( $1 \mu\text{g ml}^{-1}$  final concentration), filtered onto black,  $0.2 \mu\text{m}$  pore size, 25 mm diameter, polycarbonate membrane filter, mounted on microscope slides between layers of immersion oil. Bacteria were counted using a UV filter combination (350–360 nm excitation,  $>400 \text{ nm}$  emission) on a Nikon Microphot epifluorescence microscope at  $1,250\times$  (Porter and Feig 1980). A minimum of 300 cells was counted distributed over at least ten microscope fields (typically 20–40). Duplicate preparations were periodically counted and the coefficient of variation among replicates averaged 28% ( $n = 6$ ).

Bacterial production was measured as the incorporation of 4,5- $[\text{}^3\text{H}]$ -L-leucine into cold TCA/ethanol insoluble pools using a modification of the methods developed for water samples (Smith and Azam 1992) and sediments (Van Duyl and Kop 1994). Ten aliquots of ca.  $0.1 \text{ cm}^3$  were removed from the homogenized soil (0–1 cm layer) using a  $1 \text{ cm}^3$  syringe, placed into 2 ml pre-weighed micro-centrifuge tubes, and reweighed. The L-leucine primary stock was diluted using  $0.2 \mu\text{m}$  filtered seawater collected from Nags Creek. This working stock ( $100 \mu\text{l}$ ) was added ( $2 \mu\text{Ci}$  at  $20 \text{ Ci mmol}^{-1}$ ) to each tube and vortexed to make a soil slurry. Additional aliquots of working stock ( $20\text{--}50 \mu\text{l}$ ) were pipetted in triplicate into micro-centrifuge tubes for total activity determination. Five replicates were immediately fixed for time zero; the other five were fixed after ca. 1 h incubation ( $21^\circ\text{C}$ , dark). Several preliminary experiments established that isotope uptake was linear for the first 2 h (data not shown). Samples were fixed by addition of 1 ml of ice-cold 80% ethanol amended with a high concentration of unlabeled leucine ( $1 \text{ g l}^{-1}$ ), to dilute the  $^3\text{H}$  L-leucine specific activity and to facilitate extraction of unincorporated label. The samples were processed a total of three times by extracting in ethanol/leucine on ice for 2 h, centrifuging at  $13,000 \text{ g}$  for 5 min, and aspirating the supernatant. The tubes were placed into carrier vials with 1 ml of ScintiSafe gel cocktail and assayed for radioactivity with a Packard TR 2500 liquid scintillation analyzer using transformed spectral index quench

correction. This cocktail was designed to suspend the particles into a gel-like matrix, but soil still settled significantly, causing the measured activity to decline over time. To account for this time-dependent decline in activity, the samples were vortexed and counted twice, recording the time interval between the two counts. The activity at the time zero (the time of vortexing) was calculated by linear extrapolation. The correction was usually small (10% or less), but removed a known and variable bias. Leucine incorporation rates were scaled to carbon units using conversion factor  $3.1 \text{ kg C mol}^{-1}$  leucine incorporated, recommended by Simon and Azam (1989). Bacterial abundances and production were normalized to soil volume using a wet weight to volume density of  $0.83 \text{ gww cm}^{-3}$ .

#### Oxygen consumption, nutrient flux and denitrification

Duplicate intact cores (8 cm ID) were collected for denitrification, oxygen consumption and nutrient flux measurements from each experimental plot at low tide between July 15 and 18, 2002. Each day 8 of the 16 plots were sampled, so a total of 32 cores were used for flux measurements. Aboveground vegetation was removed by cutting stems at the soil surface prior to coring. Cores were returned to the laboratory each day and overlying water supplied from nearby Nags Creek was gently siphoned over the surface for a total volume of  $\sim 1 \text{ l}$ . An additional core with water only was incubated to account for changes occurring in the overlying water. The cores were incubated for 8 h in the dark at ambient water temperature ( $20^\circ\text{C}$ ). Overlying water samples (ca. 45 ml) were collected at 1–2-h intervals for dissolved gas ( $\text{N}_2$ , Ar, and  $\text{O}_2$ ) analysis and preserved with  $10 \mu\text{l}$  of 50% saturated solution of  $\text{HgCl}_2$ . Samples were stored underwater and analyzed within 2 weeks on a membrane inlet mass spectrometer (MIMS), as described in Kana et al. (1994). In addition to oxygen analysis by MIMS, dissolved oxygen samples (7 ml) were fixed with Winkler reagents and analyzed within 24 h by thiosulfate titration (Parsons et al. 1984). Nutrient samples collected at the same intervals as dissolved gases were filtered through GF/F filters

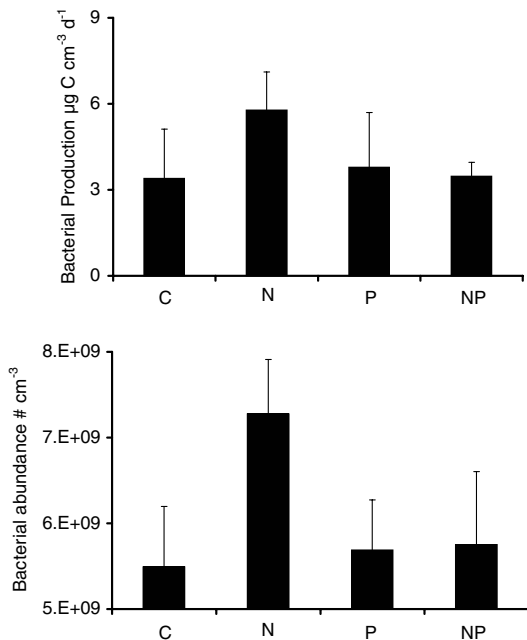
and frozen until analysis. A Lachat Instruments QuikChem 8000 FIA+ automated ion analyzer was used for  $\text{NH}_4^+$   $\text{NO}_3^-$  plus  $\text{NO}_2^-$  (hereafter referred to as  $\text{NO}_3^-$  in the text),  $\text{NO}_2^-$ , and  $\text{PO}_4^{3-}$  (referred to as DIP) analyses (Diamond 1997a, b; Huberty and Diamond 1998; Schroeder 1997). A positive flux represents release from the soil to water, while a negative flux represents uptake by the soil from the water.

#### Pore water sampling

Profiles of pore water nutrients (ammonium, nitrate, and phosphate) were sampled in situ in each plot with PVC equilibrators (Hesslein 1976; Bottomley and Bayley 1984; Wigand et al. 1997). Equilibrators (one per plot) were prepared in the laboratory using deoxygenated, deionized water in each sampling cavity and set in the field for 5 days (June 5–10, 2002) to allow for equilibration before retrieving. Dissolved constituents in the soil diffused from the soil pore water across the cellulose membrane and into the sampling cavities. Each equilibrator had six cavities, 2.5 cm in diameter every 1.2 cm on the PVC. The first cavity was near surface with the last cavity at a depth of about 20 cm. The equilibrators were sampled in the field within minutes upon removal using a syringe and filtered ( $0.8 \mu\text{m}$ , GF/F) into 20 ml vials containing  $20 \mu\text{l}$  of 6 N HCL. Water samples were transported on ice to the lab and then frozen until analysis.

#### Statistics

Bacterial abundance, soil oxygen consumption, denitrification, nutrient flux, and pore water nutrient values were natural logarithm transformed to homogenize variance prior to running a two-way ANOVA to examine for the main effects of nitrogen or phosphorus and for an  $\text{N} \times \text{P}$  interaction. The ANOVA for pore water nutrient data also included depth as a covariable. Bacterial production values were square root transformed. Differences between oxygen consumption measurements using oxygen measured by Winkler method and MIMS were compared using a paired *t*-test. Correlation analysis examined relationships among rate measurements, pore water



**Fig. 1** (a) Bacterial production as measured by leucine incorporation in  $\mu\text{g C cm}^{-3} \text{ day}^{-1}$ . (b) Bacterial abundance in  $\text{cells cm}^{-3}$ , plot mean + SE ( $n = 4$ )

concentrations and above and belowground biomass. Above and belowground biomass values are reported in Wigand et al. (2004a). The probability for significance is reported at  $p < 0.05$  for all statistical analyses.

## Results

Bacterial production in individual plots ranged from  $0.64$  to  $9.56 \mu\text{g C cm}^{-3} \text{ day}^{-1}$ . Average bacterial production was highest in the N treatment ( $p = 0.002$ ) and there was a significant  $N \times P$  interaction ( $p = 0.03$ ). The N + P treatment was less variable than the other treatments (Fig. 1a). Experimental treatments had no significant effect on bacterial abundance (Fig. 1b). The average bacterial abundance was  $8.2 \times 10^9 \text{ cells cm}^{-3}$ , ranging from  $0.5$  to  $11.3 \times 10^9 \text{ cells cm}^{-3}$  among individual plots. Bacterial production and abundance were significantly correlated ( $r = 0.65$ ).

Fertilization significantly increased all pore water nutrient concentrations (Fig. 2). Pore water  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were significantly enhanced in the nitrogen and phosphorus addition treatments and there was no  $N \times P$  interaction.

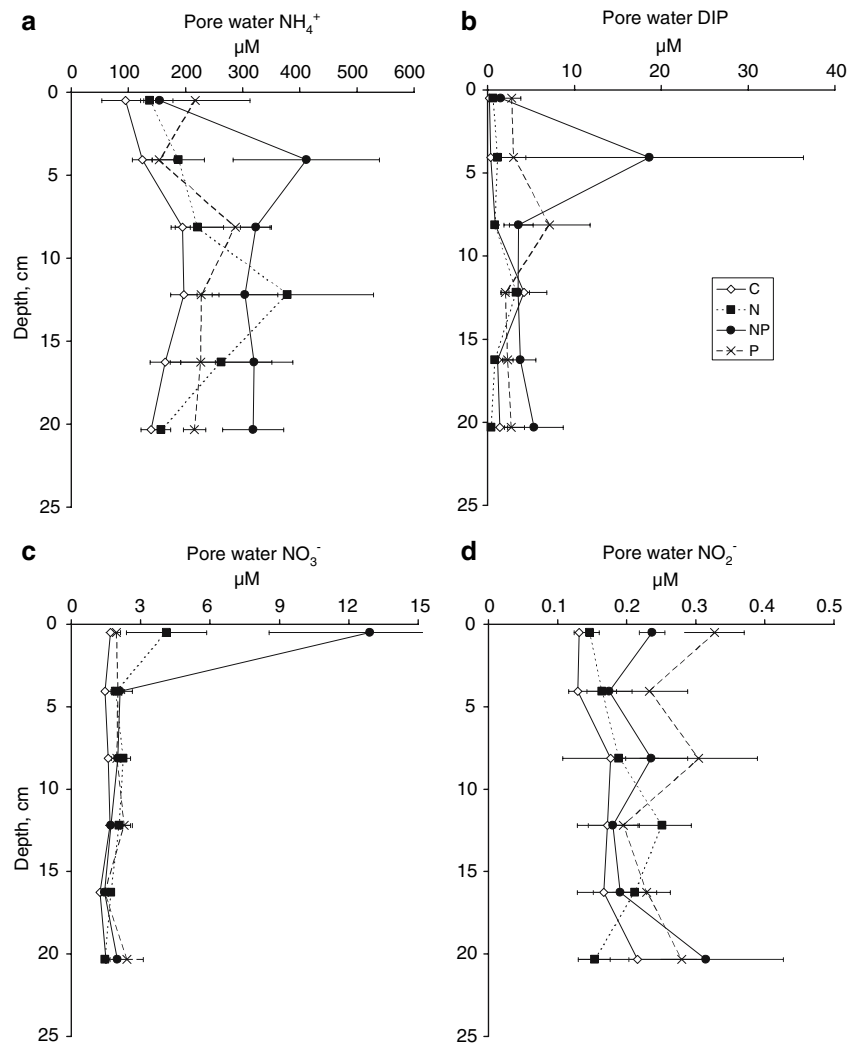
Concentrations of both nutrients were highest in N + P treatments, next highest in N-only, then P-only and lowest in control treatments. Pore water DIP and  $\text{NO}_2^-$  concentrations were significantly enhanced in the phosphorus treatments, but not the nitrogen treatments. In addition,  $\text{NO}_2^-$  concentrations had a significant  $N \times P$  interaction, while pore water DIP concentrations did not. Pore water DIP concentrations and fluxes across the marsh surface were significantly correlated ( $r = 0.81$ ).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations significantly covaried with depth, but neither DIP nor  $\text{NO}_2^-$  concentrations.  $\text{NH}_4^+$  concentrations increased with depth to a maximum at 8 cm (4 cm for the N + P treatment) (Fig. 2a). In contrast,  $\text{NO}_3^-$  concentrations were highest in the surface layer (Fig. 2c), reaching  $13 \mu\text{M}$  in the N + P treatment, higher than the concentrations found in the creek.

In general, nutrient exchange across the marsh surface was low, making it difficult to resolve statistically significant differences among the experimental treatments. Of the nutrient exchanges measured,  $\text{NH}_4^+$  flux was highest, ranging from  $-0.9$  to  $1.7 \text{ mmol m}^{-2} \text{ day}^{-1}$  in individual plots. Soil uptake of  $\text{NH}_4^+$  tended to occur in control and N-only treatments, while  $\text{NH}_4^+$  release occurred in P treatments (Fig. 3a); however these results were not statistically significant. DIP fluxes ranged from  $-0.9$  to  $0.4 \text{ mmol m}^{-2} \text{ day}^{-1}$ . DIP uptake was significantly enhanced by nitrogen (N, N + P) addition (Fig. 3b).  $\text{NO}_3^-$  fluxes ranged from  $-0.7$  to  $0 \text{ mmol m}^{-2} \text{ day}^{-1}$  in individual plots, and the experimental treatments did not significantly affect  $\text{NO}_3^-$  fluxes (Fig. 3c).  $\text{NO}_2^-$  fluxes ranged from  $-0.1$  to  $0.2 \text{ mmol m}^{-2} \text{ day}^{-1}$  in individual plots and  $\text{NO}_2^-$  uptake was significantly enhanced by P-addition (Fig. 3d).

Soil oxygen consumption rates were similar whether measured using Winkler technique or  $\text{O}_2 : \text{Ar}$  ( $p = 0.18$ ), so rates reported are the average of both techniques. The rates ranged from  $2.3$  to  $59 \text{ mmol m}^{-2} \text{ day}^{-1}$  in individual plots. Average rates in the control and +N treatment plots were  $9.3$  and  $10.7 \text{ mmol m}^{-2} \text{ day}^{-1}$ , respectively (Fig. 4a). Soil oxygen consumption was higher in phosphorus treatments than nitrogen treatments (+P =  $23.4$  and +N + P =  $31.8 \text{ mmol m}^{-2} \text{ day}^{-1}$ ) although rates were not significantly different among treatments. Denitrification rates in individual



**Fig. 2** Pore water concentrations of (a)  $\text{NH}_4^+$ , (b) DIP, (c)  $\text{NO}_3^-$ , (d)  $\text{NO}_2^-$  in  $\mu\text{M}$  with depth, plot mean  $\pm$  SE ( $n = 4$ )



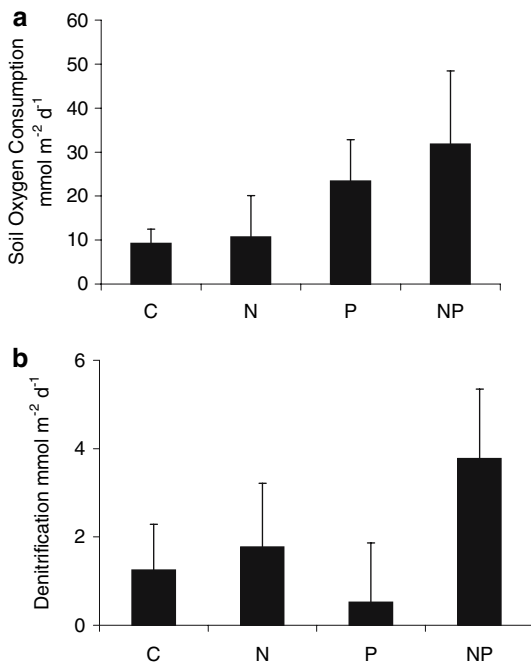
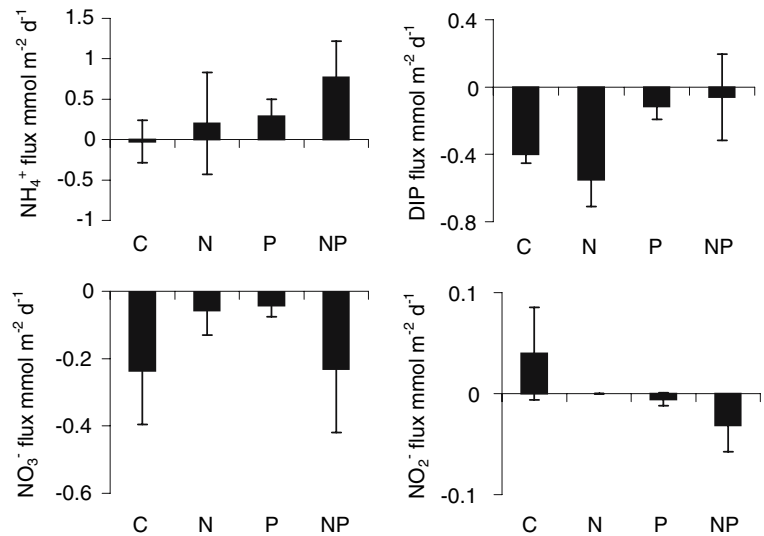
plots ranged from 0 to  $7.9 \text{ mmol m}^{-2} \text{ day}^{-1}$ , with one of the phosphorus treatments showing a net nitrogen fixation rate of  $1.3 \text{ mmol m}^{-2} \text{ day}^{-1}$ . Average denitrification rates in the control and +P treatment plots were 1.2 and  $0.5 \text{ mmol m}^{-2} \text{ day}^{-1}$ , respectively, while +N and +N + P rates were 1.8 and  $3.8 \text{ mmol m}^{-2} \text{ day}^{-1}$ , respectively (Fig. 4b). These differences among treatments were not statistically significant, due to high within-treatment variation.

## Discussion

With respect to some state and flux variables, the results in this study show clear fertilization

effects, including significant effects on bacterial production, DIP and  $\text{NO}_2^-$  nutrient fluxes, and pore water nutrient concentrations. Enhanced pore water nutrient concentrations in fertilized plots are consistent with the results from other fertilization experiments (Valiela and Teal 1974; Morris and Bradley 1999; Sundareshwar et al. 2003). For other important fluxes such as soil oxygen consumption,  $\text{NH}_4^+$  flux, and denitrification, measurements in fertilized plots were not significantly different from the control plots. Variability in rate measurements was not correlated with either above or belowground plant biomass, except for  $\text{NO}_3^-$  flux which was negatively correlated with belowground biomass ( $r = -0.75$ ).

**Fig. 3** (a)  $\text{NH}_4^+$  flux, (b) DIP flux, (c)  $\text{NO}_3^-$  flux, (d)  $\text{NO}_2^-$  flux in  $\text{mmol m}^{-2} \text{ day}^{-1}$ , positive values indicate flux out of soil, negative values indicate flux into soil, plot mean  $\pm$  SE ( $n = 4$ )



**Fig. 4** (a) Soil oxygen consumption, (b) Denitrification in  $\text{mmol m}^{-2} \text{ day}^{-1}$ , plot mean  $\pm$  SE ( $n = 4$ )

### Bacterial production and mineralization

Direct comparisons to published bacterial abundance and productivity are difficult due to different normalizing metrics (mass, volume, area). Table 1 summarizes much of the available literature on sediment bacterial abundance and productivity ranging from eutrophic freshwater

lakes to marshes to oceanic habitats, assuming nominal conversions when necessary, normalized to sediment volume. In this study, bacterial abundance averaged  $6.1 \times 10^9 \text{ cells cm}^{-3}$ , which is on the high end of the nearly 2 order of magnitude range of  $0.1\text{--}9.4 \times 10^9 \text{ cells cm}^{-3}$  reported in this literature. Similar abundances were reported in freshwater sediments (Kirschner and Velimirov 1999) and North Carolina salt marsh (Ruble and Dornseif 1978; Ruble 1982) and, somewhat surprisingly, from North Sea oceanic sediments (van Duyl and Kop 1994).

Bacterial production in this study averaged  $4.1 \mu\text{g C cm}^{-3} \text{ day}^{-1}$ , an intermediate value among a 2.5 order of magnitude range reported from the literature (Table 1). The highest productivities were reported from European lakes, while the lowest productivities were often observed in marsh soils. Bacterial turnover times ranged from less than 1 day in lake sediments (Furtado and Casper 1999) to over 200 days in salt marsh soil (Sundareshwar et al. 2003). Turnover time is affected by the quality of organic material available for bacterial metabolism and other environmental factors (e.g., temperature, redox environment). Thus, the long turnover times of 17–200 days in marsh soils suggests that the organic material fueling bacterial production is refractory compared to material available in lake, stream and seagrass sediments or microbial mats (Table 1). The stimulation of bacterial production by N-only in this experiment suggests that



**Table 1** Sediment bacterial abundance and productivities summarized from the literature

Location	Habitat	Production ( $\mu\text{g C cm}^{-3} \text{ day}^{-1}$ )		Abundance ( $\times 10^9 \text{ cells cm}^{-3}$ )		Turnover (day)	Source
		Mean	Range	Mean	Range		
North Carolina, USA	Salt marsh	ND	ND	9.4	8.4–10.9	ND	Rublee and Dornseif (1978)
North Carolina, USA	Salt marsh	ND	ND	8.5	5.0–13.8	ND	Rublee (1982)
South Carolina, USA	Salt marsh	0.1	0.07–0.1	0.9	0.42–0.94	203	Sundareshwar et al. (2003)
Georgia, USA	Fresh marsh	0.3	0.22–0.35	ND	ND	ND	Moran and Hodson (1992)
Langebaan Lagoon, S. Africa	Salt marsh	0.4	0.04–0.72	0.9	0.40–1.3	50	Tibbles et al. (1992)
NE Atlantic	Oceanic	0.6	0.06–1.23	0.2	0.06–0.41	8.1	Dixon and Turley (2001)
North Sea	Oceanic	1.4	0.64–76.8	4.9	0.79–11.0	71	Van Duyl and Kop (1994)
California, USA	Microbial mat	3.4	0.17–4.32	0.7	0.29–1.05	4.3	Hogan and Ward (1998)
Rhode Island USA	Salt Marsh	4.1	0.64–9.56	6.1	3.8–8.2	29	This study
New York, USA	Fresh marsh	4.3	0.02–22.6	3.7	0.39–6.5	17	Austin and Findlay (1989)
Pennsylvania, USA	Stream	4.7	0.44–25.5	0.6	0.18–1.9	2.6	Bott and Kaplan (1985)
Moreton Bay, Australia	Seagrass bed	9.9	0.4–53.3	0.3	0.2–0.6	0.6	Moriarty and Pollard (1981)
Vienna, Austria	Freshwater lake	21.4	1.6–59.8	4.9	2.4–8.5	4.6	Kirschner and Velimirov (1999)
Finland, southern	Freshwater lake	36	71–137	ND	ND	ND	Tuominen (1995)
Brandenburg, Germany	Freshwater lake	56.9	1.2–165	0.1	0.04–0.22	0.05	Furtado and Casper (1999)

Conversions used, when necessary: 20 fg C per bacterial cell; 3.1 kg carbon per mole leucine incorporated;  $2 \times 10^{18}$  cells per mole thymidine incorporated; 0.3 g dry sediment per  $\text{cm}^3$  wet sediment; 0.8 g wet sediment per  $\text{cm}^3$  wet sediment. Values were scaled to volume of surface sediment using nominal conversions (listed below) when necessary. Turnover times were calculated from mean abundance (scaled to carbon) and mean productivity listed in this table

bacteria are nitrogen limited. Our results contrast with the results of Sundareshwar et al. (2003), who observed P stimulation of both bacterial production and abundance in a South Carolina *S. alterniflora* marsh. The reason for this difference is unclear, although factors such as organic matter quality and quantity and the nutrient status of the bacteria are likely important.

Oxygen consumption in control treatments was low compared to rates measured in other salt and tidal fresh marshes (Christensen et al. 1981; Zelenke-Merrill 1999; Eriksson et al. 2003; Hamersley and Howes 2005). However, our rates ( $2.3\text{--}59 \text{ mmol m}^{-2} \text{ day}^{-1}$ ) are similar to those measured in other Rhode Island *S. patens* marshes ( $0.4\text{--}17 \text{ mmol m}^{-2} \text{ day}^{-1}$ ) (J. Davis, personal communication). Soil respiration was enhanced in a fertilized *S. alterniflora* marsh (Morris and Bradley 1999). Nutrient fluxes were on the low end of the range, particularly  $\text{NH}_4^+$  fluxes compared to other studies (Eriksson et al. 2003; Davis et al. 2004). Pore water nutrient concentrations were generally comparable (Bowden 1986; Scudlark and Church 1989; Langis et al. 1991; Chambers et al. 1992; Howes and Goehring 1994),

although  $\text{NO}_3^-$  concentrations can be elevated due to groundwater inputs (Anderson et al. 1997; Tobias et al. 2001). Enhanced pore water nutrient concentrations in fertilized plots are consistent with the results from other fertilization experiments (Valiela and Teal 1974; Morris and Bradley 1999; Sundareshwar et al. 2003). The shape of pore water profiles suggest that  $\text{NH}_4^+$  and DIP were consumed at the 5 cm level and above where most of the roots and rhizomes of *S. patens* are located (Valiela et al. 1976). This indicates that much of the  $\text{NH}_4^+$  produced in soils through mineralization was removed through processes such as plant uptake and nitrification.

#### Denitrification rates

$\text{NO}_3^-$  concentrations were low ( $<5 \mu\text{M}$ ) in the tidal creek near this site during the sampling period, similar to other measures of summer  $\text{NO}_3^-$  concentrations in this area, which averaged less than  $2 \mu\text{M}$  (K. Raposa, personal communication, C. Wigand unpublished data). Thus, the water from the creek was an insignificant source of  $\text{NO}_3^-$  for denitrification and most of the

**Table 2** Denitrification rates in tidal marsh sediments after Valiela et al. (2000)

Producer taxon associated with measurement	Location	Denitrification rate (mmol N m <sup>-2</sup> day <sup>-1</sup> )	Method	Reference
<i>Spartina alterniflora</i>	MA	0.7–6.0	N <sub>2</sub> /Ar gas partitioning	Kaplan et al. (1979)
<i>S. alterniflora</i>	MA	6.7	N <sub>2</sub> flux	Johnson et al. (1994)
<i>S. alterniflora</i>	MA	1.8	<sup>15</sup> N tracer	White and Howes (1994)
<i>S. alterniflora</i>	MA	2.1	<sup>15</sup> N tracer	Hamersley and Howes (2005)
<i>S. alterniflora</i>	VA	0.1	N <sub>2</sub> /Ar	Anderson et al. (1997)
<i>S. alterniflora</i>	NC	0.05–0.3	Acetylene	Thompson et al. (1995)
<i>S. alterniflora</i>	GA	2.4	N <sub>2</sub> sediment profiles, diffusion model	Haines et al. (1977)
<i>S. alterniflora</i>	GA	13	N <sub>2</sub> O reductase	Whitney et al. (1981)
<i>S. alterniflora</i>	GA	10–99	Acetylene	Dollhopf et al. (2005)
<i>S. alterniflora</i>	LA	0.6	<sup>15</sup> N	DeLaune and Patrick (1980)
<i>Spartina patens</i> and <i>Distichlis spicata</i>	MA	0–1.9	N <sub>2</sub> /Ar gas partitioning	Kaplan et al. (1979)
<i>S. patens</i>	LA	0.3	Mass balance	DeLaune and Patrick (1990)
<i>S. patens</i>	RI	–2.9–8.0	N <sub>2</sub> flux	Davis et al. (2004)
<i>S. patens</i>	RI	1.2	N <sub>2</sub> /Ar	This study
<i>Limnolium serotinum</i>	Italy	0.2–6.2	<sup>15</sup> N	Erkisson et al. (2003)
<i>Juncus maritimus</i>	Italy	0.7	<sup>15</sup> N	Erkisson et al. (2003)
<i>Halimione portulacoides</i>	Italy	0.7	<sup>15</sup> N	Erkisson et al. (2003)
<i>Puccinellia maritima</i> (Huds.) and <i>Halimione portulacoides</i> (L.)	UK	0.1–0.3	<sup>15</sup> N tracer	Abd. Aziz and Nedwell (1986)
<i>H. portulacoides</i>	UK	0.1–1.4	Acetylene	Koch et al. (1992)
<i>Salicornia virginica</i>	CA	3.4	Acetylene	Joye and Paerl (1994)
<i>Trapa natans</i> (L.)	NY	3.1	N <sub>2</sub> /Ar	Zelenke-Merrill (1999)
<i>Nuphar advena</i> (Ait.)	MD	3.1	N <sub>2</sub> /Ar	Merrill and Cornwell (2000)
<i>Zizania aquatica</i> L.	MD	2.4	N <sub>2</sub> /Ar	Merrill and Cornwell (2000)

NO<sub>3</sub><sup>-</sup> needed for denitrification must have come from nitrification in the soils. The peak NO<sub>3</sub><sup>-</sup> concentrations in the surface soil layer, up to 13 μM in the N + P treatment, are further evidence supporting high nitrification rates in these soils. Assuming all of the NO<sub>3</sub><sup>-</sup> taken up by soils was denitrified, the overlying water NO<sub>3</sub><sup>-</sup> could supply 19% of denitrification in the control plots, but only about 5% for the fertilization treatments. Denitrification rates were comparable to those measured in other tidal marshes (Table 2). Most denitrification studies have shown that rates in marshes are limited by NO<sub>3</sub><sup>-</sup> supply either from the overlying water (Abd. Aziz and Nedwell 1986; Lindau and DeLaune 1991; Koch et al. 1992; Seitzinger 1994; Merrill and Cornwell 2000; Eriksson et al. 2003) or groundwater (Tobias et al. 2001) and that nitrification rates are low (Abd. Aziz and Nedwell 1986). However, high nitrification rates have been measured (Kaplan et al. 1979; Bowden 1986; Thompson et al. 1995; Eriksson et al. 2003; Dollhopf et al. 2005), or high potential for nitrifi-

cation based on pore water profiles and benthic fluxes in high *S. alterniflora* marsh (Scudlark and Church 1989). Although we did not measure nitrification, peak pore water NO<sub>3</sub><sup>-</sup> concentrations are consistent with nitrification in the surficial soil layers (Fig. 2c).

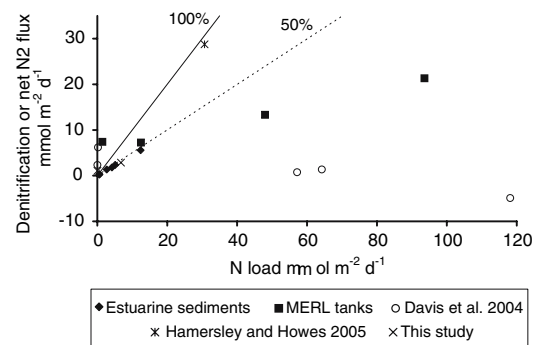
Although denitrification rates measured in intact cores showed no fertilization effects, denitrification enzyme activity measured in soil slurries from these same plots in June in a concurrent study showed significantly elevated rates in the N treatments (Wigand et al. 2004b). This suggests that with sufficient nitrate and labile organic matter, added nitrogen enhances denitrification rates. We may not have observed differences in the denitrification rates in this study because of competition for nitrogen between plants and denitrifiers during the peak of the growing season.

Previous studies have shown variable biogeochemical and microbial responses to fertilization. In one of the few studies to measure denitrification in intact marsh soils, Kaplan (1977) and

Hamersley and Howes (2005) observed a significant stimulation in fertilized treatments compared with controls. Denitrification rates in both control and fertilized plots increased rapidly between June and July in a *S. alterniflora* marsh, with higher rates in fertilized than control plots (Hamersley and Howes 2005). Rates in these plots remained high until October when temperature decreased (Hamersley and Howes 2005). Similarly, increased N loads led to increased denitrification in *S. alterniflora*, such that two thirds of added N was denitrified (Teal and Howes 2000). The effect of fertilization was different between *S. alterniflora* and *S. patens* marshes, with higher denitrification rates in *S. alterniflora* than *S. patens* (Kaplan 1977). Measurements of net N<sub>2</sub> fluxes in intact *S. patens* cores along a gradient of nitrogen loading in Narragansett Bay showed an initial increase in denitrification at low loading rates (ca. 0.2 mmol m<sup>-2</sup> day<sup>-1</sup>), but a decrease at higher loading rates (Davis et al. 2004; Fig. 5). Studies in estuarine sediments, suggest that denitrification can remove ~50% of the nitrogen load to the estuary (Seitzinger 1988). As loading rate increases above 50 mmol m<sup>-2</sup> day<sup>-1</sup>, denitrification removes a smaller percentage of the load, with marshes being much less efficient than estuarine sediments (Fig. 5). In this study denitrification was able to remove about 43% of the nitrogen added in the fertilization treatments. Further experiments examining fertilization effects at intermediate loading rates are needed to determine what nitrogen load gives the peak denitrification rate and why denitrification appears to decline at high loading rates. Perhaps greater mineralization of organic carbon with increased nutrient loading (Morris and Bradley 1999) led to enhanced activity by some heterotrophs but not others (Sundareshwar et al. 2003).

#### Comparisons between *Spartina patens* and *Spartina alterniflora*

Relatively little research on biogeochemical and microbial processes has been done in *S. patens* marshes compared to *S. alterniflora* marshes. There are important differences in physiology and elevation (or flooding frequency) between these marsh types that likely affect biogeochemical



**Fig. 5** Denitrification or Net N<sub>2</sub> flux (mmol m<sup>-2</sup> day<sup>-1</sup>) versus nitrogen load (mmol m<sup>-2</sup> day<sup>-1</sup>). Data from Seitzinger and Nixon (1985) (MERL tanks) Seitzinger (1988) (estuarine sediments), Davis et al. (2004), Hamersley and Howes (2005), and this study. 50 and 100% lines indicated by dashed and solid lines, respectively. Positive values indicate net denitrification, negative net nitrogen fixation

processes. One physiological difference is that *S. patens* supports endomycorrhizal symbionts, while *S. alterniflora* does not. In the present study, P fertilization was expected to inhibit fungal symbionts associated with the *S. patens* roots because the plants would have a direct source of P. While there was no significant difference in fungal symbionts among treatments (Wigand et al. 2004a), we observed a significant reduction in P uptake by soils in the P treatments (P-only and N + P). This is consistent with plant P demand being satisfied by fertilizer additions rather than through the endomycorrhizae. In addition to the direct connection between endomycorrhizae and plant P demand, the endomycorrhizae can fuel N-fixation indirectly by facilitating the release of organic exudates, which N-fixing bacteria can use (Burke et al. 2002b). Therefore, elevated P might also inhibit the N-fixers associated with the roots that relied on the mycorrhizal association (Burke et al. 2002b). In addition, the P would stimulate the competition between the bacterial mineralizers, N-fixers, and denitrifiers for organic carbon.

*S. patens* soils in New England are only saturated during high tides in contrast to *S. alterniflora* marshes, which are flooded each high tide. Nitrification could be enhanced throughout soil column on ebb tides as *S. patens* marshes drain, while denitrification would be enhanced when soils are saturated and more anaerobic. In addition, evapotranspiration by plants increases water

uptake leading to enhanced oxidation of the soil (Dacey and Howes 1984), which could also enhance nitrification rates. An important physiological difference between *S. alterniflora* and *S. patens* is root ventilation by *S. alterniflora* but not *S. patens* (Gleason and Zieman 1981; Howes and Teal 1994). This suggests that *S. alterniflora* has a greater potential to oxidize the rhizosphere than *S. patens* (Howes and Teal 1994). In submersed plants, those with high root oxygen release have enhanced potential nitrification and denitrification compared to those with low root oxygen release (Caffrey and Kemp 1990), although rates in intact sediments vary throughout the growing season (Caffrey and Kemp 1992). Thus, while enhanced rhizosphere oxidation by *S. alterniflora* may enhance nitrification and denitrification compared to *S. patens*, other processes promoting physical transport of oxygen may also be important.

## Conclusions

*Spartina patens* marshes located along the upland border have the potential to intercept and transform anthropogenic nutrient inputs before they reach the estuary, thus ameliorating the effects of eutrophication. In this study, we have shown that fertilization, particularly nitrogen fertilization, stimulates bacterial processes, enhances pore water nutrient concentrations and alters some nutrient fluxes across the marsh surface. The effect of fertilization on mineralization and nitrogen removal through denitrification was inconclusive. Further research examining seasonal changes in denitrification rates would allow us to determine how effective *S. patens* marshes are at removing excess nutrient inputs.

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